Studies on Crude and Active Ingredients of Ethanolic and Hydroethanolic Extracts of *Prosopis Juliflora*

A Synopsis

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INTRODUCTION

Medicinal plants play an important role in meeting the medical and health needs of about 70% of people in industrialized and developing countries who are an important resource for the treatment of various diseases and diseases (Ngari *et al.,* 2010). Medicinal plants are rich in antimicrobial agents. The plants are used medically in different countries and are the source for powerful and highly effective drugs (Srivastava *et al*., 1996). According to the World Health Organization (WHO), more than 80% of the world's population depends on traditional medicine for their needs in primary health care (Thomson, 2010). Herbal plants are traditionally used to treat various diseases in India and around the world since the beginning of civilization. In fact, natural products are a source of synthetic and traditional herbal medicine. Traditional plants have long-term remedies for human diseases because they contain ingredients, Therapeutic value and some of them are also used prophylactically (Nostro *et al*., 2000). Herbal treatment of any plan that contains one or more substances that can be used for therapeutic or precursors for the synthesis of drug objects. The medicinal value of these plants is in certain chemicals that produce a physiological effect on the human or animal body (Edego *et al*., 2005). Herbal plants have various pharmacological activities, such as anti-inflammatory, antiulcer, antibacterial, antioxidants, cancer, antiurolithiatic (Moteriya *et al.,* 2015) Bioactive molecules which act as antioxidants and antimicrobial(Sengul *et al*., 2009). Herbal remedies can protect the human body from cell oxidation reactions and through various pathogen-causing infections (Wojdylo *et al*., 2007). Microorganisms have the potential to cause a disease. The discovery of antibiotics in the early twentieth century provided an increasingly important bacterial disease to combat. However, due to arbitrary use of commercially available antimicrobial agents commonly used in the treatment of infectious diseases, it has developed multi-drug resistance. (Davis, 1994 and Ahamad *et al*., 1998). In addition to this problem, antibiotics are sometimes associated with adverse effects on the host, including hypersensitivity, immunosuppression and allergic reactions (Monroe and Polk. 2000). This creates a need for new, effective and safe antimicrobial agents. In this regard, naturally occurring medicinal plants provide with active ingredients that exhibit antimicrobial activity, the broad field of research. Anti plant microbial plants have tremendous therapeutic potential. They are effective in treating infectious diseases and at the same time reduce many of the side effects commonly associated with synthetic antimicrobial agents. The beneficial medical effects of plant materials typically result from the combinations of the

Secondary products that are present in the plant. In plants of these compounds are secondary metabolites, mainly such as alkaloids, steroids, TAN-Nine and phenolic compounds, flavonoids, steroids, resins, fatty acids, which are capable of generating a defined physiological effect in the body (Agrawal., 1996). Secondary metabolites are chemical compounds during the normal metabolic processes of the plant formed and plants they use to protect themselves. (Alison *et al* 2001, Ning *et al.,* 2009). Free radicals are important mediators that cause inflammation and neutralize by antioxidants that exert anti-inflammatory effects (Filomena *et al.,* 2008) Free radical scavenging molecules such as flavonoids, tannins, alkaloids, quinones, amines, vitamins and other metabolites have anti-inflammatory, anti-carcinogenic, anti-bacterial and anti-viral activity (Sala *et al.,* 2002). Most phytochemicals have antioxidant activity and protect cells against human oxidative damage. Plants with antioxidants are used to minimize the severity of diseases related to inflammation, and it is believed that due health benefits of plant antioxidants in their protective effect by counteracting action of ROS (Wong *et al., 2006*). The first definition of antioxidant was by Halliwell In 1989 as "any substance that inhibits the oxidation of such substrates delayed significantly at low concentrations compared to oxidizable substrates (carbohydrates, lipids, proteins or nucleic acids) or" (Halliwell and Gutteridge., 1992). Later, other definitions of antioxidants have been proposed as "any substance that prevents oxidative damage to a target molecule, either deferred or eliminated" (Halliwell and Gutteridge., 1990). or "any substance can remove reactive oxygen species directly or indirectly and as a regulator Of the antioxidant defenses or inhibit the production of these species" (Khlebnikov *et al*., 2007). People have developed antioxidant systems to protect against free radicals. These systems include some products in food antioxidants (exogenous) of the body (endogenous) and others. The first comprises a enzymatic defense mechanisms such as glutathione peroxidase, catalase and superoxide dismutase, superoxide, hydrogen peroxide and lipid peroxides tabulate thus the formation of toxic OH and b) non-enzymatic repellents, such as Glutathione, histidine peptides, iron binding to transferrin and ferritin proteins, dihydrolipoic acid, melatonin, uric acid and Plasmaprotethiol (Sharma and Agarwal., 2004, Kunwar *et al*., 2011). Isolation and identification of bioactive compoundss present in a crude extract sample Serve as a building block for the development of new types of therapies with new the treatment of the mechanisms of action with a stronger species Several human diseases (Lee *et al*., 2000). The plant extracts are usually produced as a combination of different types of biologically active compounds or

phytochemicals of different polarity, the separation remains an important procedure for the identification and characterization of bioactive compounds. It is common to use pure compounds to isolate these bioactive compounds as a number of different separation techniques such as TLC, column chromatography, flash chromatography, HPLC and Sephadex.

The plant chosen for this study is *Prosopis juliflora.* The genus *Prosopis* belongs to the legume family (*Mimosaceae*). Approximately 45 species of this genus are known. The genus *Prosopis* has many uses in folk medicine (Saidman and Vilardi 1987, Nadeem, 1992). This plant is found in the arid and semi-arid regions of India. It has been used as a popular remedy for colds, colds, diarrhea, dysentery, eccentric, influenza, inflammation, measles, sore throat, and for wounds (Singh.S, 2012). It has several properties that are useful to the human species. Each part of *P.juliflora* is used abundantly in various fields. Research into the development of *P. juliflora* by its Alleopathy, pharmaceuticals and bio-pesticide will have a major impact on the development of new drugs and pesticides. (Dave and Bhandari., 2013). The allelopathy is one of the interesting characteristics that in some plants like Prosopis sp. In addition, several studies have reported the presence of allelopathic compounds in *P. juliflora.* (Nakano *et al.*, 2001) that L-tryptophan may play an important role in leaf allelopathy of *P. juliflora* (Kaur *et al*., 2012), which demonstrate the L-tryptophan in leaf leachates of *P. juliflora*.

REVIEW OF LITERATURE

Srivastava *et al.*, (1996) investigated in his work the medicinal plants are rich sources of antimicrobial agents. Plants are used medicinally in different countries and are the source of potential and powerful drugs.

Adenisa *et al.*, (2000) found that the bacterial infections have become widespread due to various factors, such as the HIV / AIDS pandemic, lack of hygiene, overcrowding and resistance to conventional antimicrobials. Higher natural plant products may provide a new source of antimicrobial agents with novel mechanisms of action.

According to Nwachukwe and Umechurma, (2001) studied in his research the medicinal plants would get the best source of a variety of drugs. In recent years, attention has been paid to natural treatment methods for protection and management against pathogens. Plant extracts have played a significant role in inhibiting pathogens and improving food quality and yield.

Choge *et al*,. (2002) studied that *Prosopis juliflora* was introduced in 1973 for the rehabilitation of quarries in Kenya and the preservation of existing natural vegetation against overloads. Over the years, the Prosopis however, has expressed in recent years, what natural habitats, areas and crops in many parts of the country adversely affected.

According to Lakshmi *et al.*, (2010) investigated that the alkaloids present in the plant leaves can also be used as a lead bio-pesticide in combating the diseases caused by several phytopathogens on cereal crops (Seetha Lakshmi et al., 2010).

Devendra *et al.*, (2011 found that the antimicrobial activity of *Moringa oleifera* Lam., Leaf extract, chloroform extract of plant leaves antibiotic properties against a wide range of pathogens such as *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424) (ZOI = 9.5 ± 0.5 mm), *Staphylococcus aureus* (MTCC3160) (ZOI = 6.2 ± 0.7 mm) *Streptococcus pyogenes* (MTCC 442) (ZOI = 7.0 ± 0.5 mm). *Aspergillus niger* (MTCC 1781) (ZOI = 7.3 to 0.5 mm), *Candida albicans* (MTCC 181) (ZOI = 6.2 ± 0.5 mm), together with positive controls. This extracts plants with good healing properties without side effects compared to synthetic antibiotics.

Napar *et al.*, (2012) studied the Antimicrobial and antioxidant activities of Mimosaceae plants; *Acacia modesta* Wall (Phulai), *Prosopis cineraria* (Linn.) and *Prosopis juliflora* (Swartz). The extracts of methanolic leaf of three species of the family Mimosaceae. They were used to evaluate their antifungal and anti-bacterial antioxidant activity. The extracts were tested against four bacterial strains (*Bacillus subtilis*, *Escherichia coli*, *Vibrio cholerae* and *Enterobacter aerogenes*) and two strains of fungi *(Aspergillus niger and Aspergillus fumigatus*) tested. At 15 mg / ml concentration of the extract were observed the zones of maximum inhibitor in *Acacia modesta, P. cineraria* and *P. juliflora*, 20, 18 or 25 mm. *P. cineraria* gave the best response against *A. niger* and *A. fumigatus*, producing inhibition 15.38 or 8%. *P. juliflora* showed 7.69% inhibition against *A. niger*. The antioxidants these medicinal plants showed significant results. A maximum activity of free radicals of the treasure (% of BSA) in *P. cineraria* and *P. juliflora*, that is, 60.48 or 47.82% in comparison with *A. modesta* was observed, obtaining a minimum value of RSA of 41.42%. The three plants Mimosaceae were most effective against all types of microbes.

Ibrahim *et al.*, (2013) investigated the phytochemical analyes of *Prosopis juliflora* Swartz DC **.** The results showed the quantitative analysis of the following classes of natural ingredients: flavonoids, alkaloids, saponins, phenols and tannins in the range of (16 ± 0,39), (3,6 ± 0,06), (2,2 ± 0.23), (0.66 ± 0.11) and (0.33 ± 0.07)%. The content of fiber and crude ash was also analyzed and found (17.5 ± 0.14) and (9.5 ± 0.08)%, while pectin was calculated as (4.9 ± 0.18)%. In the continuation of the current studies more analyzes and investigations were carried out already has been with the extraction and chromatography for the isolation and purification of compounds of criminal origin in flavonoids and alkaloids.

Thakur *et al., (*2014) studied the Evaluation of antibacterial activity of *Prosopis Juliflora* (sw.) Dc. Leaves. Concluded that the extracts of this plant have antibacterial potential against cultures of target-type bacteria. Qualitative phytochemical screening of the raw extracts confirmed that this plant is a rich source of active chemical ingredients. The antibacterial activity of the crude extracts and various fractions may be due in large measure to the cumulative effect of existing phytochemistry. The absence of anthraquinones and saponins in all crude extracts implies that these compounds have no role in their overall antibacterial activity. Additional studies should emphasize the isolation and characterization of bioactive compounds and complete it is performed in vitro and in vivo to shore up the selection of active and non-toxic antibacterial

phytoconstituents which may lead to the formulation of new antibacterial drugs. On the other hand, this study provides a scientific basis for the development of new, safe and clinically effective medicine.

Tajbakhsh *et al*., (2015) studied the Invitro Antibacterial Activity of the *Prosopis Juliflora* Seed Pods on Some Common Pathogens. *P. juliflora* seed pods from Bushehr, south west of Iran could be a suitable natural source of antibacterial ingredients and is a suitable candidate for the purification of crude extracts and the next in vivo studies.

Kapoor *et a*., (2015) concluded the Antimicrobial activity of different herbal plants extracts contain antimicrobial effect of herbal extracts of some parameters such as the plant material, the technique, the growth medium and, in particular, the microorganism to be tested is dependent. For a better search for a better quality of plant material is selected. The solvent and the extraction system may either change the final result.

Nnanga *et al*., (2016) investigated the Phytochemistry and *in vitro* Antimicrobial, Antioxidant Activities of *Entandrophragma candollei* H. The research was carried out to investigate the antimicrobial and antioxidant activity in vitro of *E.candollei* extract (family *Meliacea*e). Disc-diffusion technique has been used for antibacterial and antifungal in vitro screening. The minimum inhibitory concentration of the promising extracts was determined by the broth microdilution method. Phytochemical screening showed the presence of Flavonoids compounds, tannins and saponins. The Inhibitions diameters were ranging from 7 to 17 mm and minimum inhibitor concentrations (MICs) from 156.25 to 2500 ug / ml Ethyl acetate and ethanol extract showed a high activity of finding objects with IC_{50} in the Range from 9.1026 to 11.8298 Pm. G / ml The ferric-reducing feed activity has an active concentration in the range of 12.5-200 g / ml obtained. These results suggest that E. extracts possess antibacterial activity and antioxidant *candollei* and therefore justify their use in traditional medicine for the treatment of various diseases.

Objectives

1. To evaluate the Phytochemical investigation of ethanolic and hydroethanolic extracts of *Prosopis Juliflora.*
2. To evaluate the Anti-microbial activity of ethanolic and hydroethanolic extracts of *Prosopis Juliflora*.
3. To evaluate the *in vitro* Anti-oxidative potential of ethanolic and hydroethanolic extracts of *Prosopis Juliflora.*
4. To isolation, purification and identification of active principle compounds from the extract showing the anti-oxidative potential.

Methodology

- **Procurement of plant material**

The leaves, flowers, pods and bark of *Prosopis Juliflora* procured from Khejari Nursery, Jaipur, and Rajasthan, India. The plant was used by available literature and authenticated by Biotechnologist Dr. Swaati Sharma, Assistant Professor Dept. of Agriculture, Food & Biotechnology Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan.

- **Preparation of hydro-ethanolic crude extracts by Direct Soxhlet method**

The plants parts will be washed with tap water and air drying in the shade for about 2-3 weeks. The dry parts are more ground to give a thick powder and sieved through sieve no. 80 and stored in airtight containers in the refrigerator. 25 grams of coarse ground powder from each part of the plant will be carefully packed in a filter paper thimble neatly and defatted, then extracted into 250-300 ml. of ethanol & hydro ethanol (1:1) by hot continuous percolation using the Soxhlet apparatus and heated for an additional period of 18 to 24 hours at 70°C under reflux. The collected liquid extracts will be evaporated on a rotary evaporator. The reduced extracts will be collected on glass Petri dishes and evaporated, forming semisolids. This hydroethanolic extracts will be labeled and preserved in desiccators with $CaCl_2$. (Garg *et al*., 2014)

Plant Part	**Name of the Ethanolic and Hydro-ethanolic extract**
Prosopis juliflora Leaves	PEL and PHEL
Prosopis juliflora Flower	PEF and PHEF
Prosopis juliflora Pod	PEP and PHEP
Prosopis juliflora Bark	PEB and PHEB

Ethanolic and Hydro-ethanolic extracts of various parts of *Prosopis Juliflora.*

- **Phytochemical Screening**

Qualitative tests will be undertaken on the hydro-ethanolic extracts of aerial parts of *Prosopis Juliflora* using standard protocols for the identification of phytochemicals (Harborne, 1973; Trease and Evans, 1989; Safowra, 1993; Edeoga, 2005).

- Test for Alkaloids
- Test for Fatty Acids
- Test for Flavonoids
- Tests for Glycosides
- Test for Phenols
- Test for Resins
- Test for Terpenoids (Salkowski test)
- Test for Saponins
- Test for Steroids
- Test for Tannins
- Test for Phlobatanins
- Test for Triterpenes
- Test for cardiac glycoside (Keller-Killani test)

➢ **Antimicrobial Activity (Selvamohan *et al*, 2012)**

- Micro organisms used: Standardized strains of Bacterial and Fungal.
- Assay of antimicrobial activity using Disc diffusion method.
- Antimicrobial activity of commercially available antibiotics.

➢ **Methods Employed for *In-Vitro* Anti-Oxidant Activity:** All extracts are analyzed for antioxidant potential by the following methods-

Free Radical Scavenging Potential

ABTS (2,2'-azinobis[3ethylbenzthiazoline]-6-sulfonic acid) free radical scavenging activity **(Roberta *et al,* 1999)**

➢ ***In Vitro* Tests for Enzymatic Antioxidants**

- Superoxide Dismutase (SOD) activity (Beauchamp and Fedovich, 1976)
- Peroxidase activity (Bania And Mahanta, 2012)
- Catalase (CAT) activity (Chance and Maehly, 1995)
- Glutathione Peroxidase (GPx) activity (Starlin and Gopalakrishnan, 2013)

➢ ***In-Vitro* Assay of Non-Enzymatic Antioxidants**

DPPH Radical Scavenging Assay

The free radical scavenger activity was then determined by calculating the Inhibition percentage (I) as follows. I= (Abs control – Abs sample) / Abs control X 100

Metal Chelating Activity

The percentage inhibition of Ferrozine– Fe^{2+} complex was calculated using the formula:

I= (Abs control – Abs sample)/Abs control X 100

Hydroxyl Radical Scavenging Assay

The percent inhibition (I) of Desoxyribose degradation was calculated using the formula.

I= (Abs control – Abs sample) / Abs control.

Ascorbic acid was used as a positive control.

Isolation, purification and identification of active principle compounds from the extract showing the anti-oxidative potential:

Isolation and purification was performed by Thin Layer Chromatography (TLC) and Column Chromatography were performed for separating the principle components from the best plant part for (antimicrobial), (anti-diabetic and anti-oxidative) activity and the isolated compound was characterized by HPLC, FTIR, NMR and GC-MS techniques (Sharma and Garg, 2011).

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